PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Marco CATTARUZZA and Markus HECKER	Group Art Unit: 1635
Serial No.: 10/527,785	Examiner: Louis Wollenberger
Filed: March 11, 2005	Atty. Dkt. No.: DEBE:053US
For: FUNCTIONAL CORRECTION OF THE 786C/T-VARIANCE OF THE HUMAN eNOS-GENE	Confirmation No.: 1068

CERTIFICATE OF ELECTRONIC SUBMISSION
DATE OF SUBMISSION: November 29, 2007

DECLARATION OF MARKUS HECKER UNDER 37 C.F.R. §1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

I, Dr. Markus Hecker, do declare that:

1. I am a citizen of Germany residing at Heidelberg. I currently hold the position of Full Professor and Chairman at the Institute of Physiology and Pathophysiology of the University Hospital Heidelberg. My research experience includes well over 100 original articles in peer reviewed international scientific journals and close to 40 review articles in scientific journals, journal supplements, conference proceedings and books. I have

trained in Biology, Biochemistry, Pharmacology and Physiology and hold several university degrees including a doctorate in Biochemistry and a state doctorate in Physiology. I have worked in cardiovascular research for almost 20 years, mainly focusing on molecular and cell biology issues in vascular cells. I have a special expertise in the analysis and therapeutic manipulation of transcription factors and in this capacity have been the inventor of 69 patent applications of which 9 have been granted. A copy of my *curriculum vitae* is attached.

2. The findings revealed in the aforementioned patent application (of which I am an inventor) as well as in the two related publications M. Cattaruzza et al., Circ Res. 95, 841-847, 2004 and I. Melchers et al., Arthritis Rheum. 54, 3144–3151, 2006 (of which I am the senior author and a co-author, respectively) of a higher risk of individuals homozygous for the T786C polymorphism of the human endothelial nitric oxide synthase (*nos-3*) gene for contracting coronary heart disease as well as rheumatoid arthritis are generally applicable to atherosclerosis. Atherosclerosis is a systemic and chronic inflammatory disease of the vessel wall of large conductance as well as small resistance-sized arteries (and arterioles) that may also present as transplant atherosclerosis, especially in solid organ transplants such as the heart, venous bypass graft vasculopathy and restenosis following angioplasty. The common denominator of both the classical type of atherosclerosis and its aforementioned variants is endothelial dysfunction, commonly referred to as a decreased bioavailability of endothelial cell-derived nitric oxide resulting in an exaggerated endothelial cell-leukocyte interaction and leading to chronic inflammation (excerpt from Cattaruzza et al. 2004: "Although cellular events leading to

the formation of coronary atherosclerotic lesions are not yet fully characterized, persistent dysfunction of the endothelium in affected arteries is an important aspect of this chronic inflammatory disease." And further: "The decreased capacity of the endothelium of CC carriers to generate NO is likely to promote the early phase of atherosclerosis and, as a consequence, accelerate plaque formation not only in the heart but also at other clinically important sites."). Depending on the location in the vasculature, atherosclerosis manifests itself as coronary artery or coronary heart disease which in the majority of cases leads to myocardial infarction and subsequently to heart failure. Atherosclerosis in the cerebral vasculature results in the majority of cases in stroke or multi-infarction dementia while in the periphery, especially in the arteries of the leg, it causes peripheral artery disease. Therefore applicable diseases for which the decoy oligodeoxynucleotides disclosed in the relevant patent application may be used for as drugs encompass atherosclerosis in general together with its manifestations coronary heart (artery) disease, cerebrovascular disease and peripheral artery disease as well as the sequelae myocardial infarction and heart failure, stroke and multi-infarction dementia and gangrene, respectively. Indications for which the disclosed treatment modality is equally suited include transplant atherosclerosis or vasculopathy (chronic rejection), venous bypass graft atherosclerosis or vasculopathy and restenosis following angioplasty. In addition, rheumatoid arthritis and closely related chronic inflammatory diseases which like atherosclerosis can be traced back to endothelial dysfunction (excerpt from Melchers et al. 2006: " In the last decade, the notion of RA as a leukocyte-mediated disease characterized by autoimmune reactions has been confirmed in many details. However, this view of the pathogenesis of RA has by-and-large obscured the role of a functional endothelium in the disease. In contrast,

evidence is mounting that patients with RA often develop endothelial dysfunction, possibly due to the presence of multiple proinflammatory stimuli. This may also provide an answer to the related question of whether RA itself constitutes a risk factor for atherosclerosis. In fact, patients with manifest RA have an increased risk of dying prematurely from cardiovascular disease as compared with the general population."), are amenable to treatment with the disclosed decoy oligodeoxynucleotides.

3. I declare that all statements made herein of my own knowledge are true, and that all statements of my own belief are believed to be true, and further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under § 1001 of title 18 of the United States Code.

Heidelberg, 11-22-2007

Date

Dr. Markus Hecker

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Born:	04 January 1960

Scientific curriculum

1980-1985	Study of Biology at the University of Konstanz, Germany
1985	Diploma (M. Sc. in Biology), University of Konstanz
1985-1987	Postgraduate studies at the University of Konstanz
1988	Dr. rer. nat. (Ph. D. in Biochemical Pharmacology), University of Konstanz
1988-1989	Visiting scientist, Department of Physiology and Biophysics, Georgetown University, Washington, D.C., U.S.A
1989-1990	Visiting scientist, William Harvey Research Institute, St. Bartholomew's Hospital Medical College, London, U.K.
1990-1991	Senior Scientist and Honorary Lecturer, William Harvey Research Institute, London
1991-1993	Lecturer, Department of Applied Physiology, University of Freiburg, Germany
1993	State doctorate (Dr. rer. nat., habil. in Physiology), University of Freiburg
1993-1996	Assistant Professor, Department of Cardiovascular Physiology, University of Frankfurt/M., Germany
1996-2004	Professor (C3) and Head, Department of Cardiovascular Physiology, University of Göttingen, Germany
2004 –	Professor (C4) and Director, Institute of Physiology and Pathophysiology, University of Heidelberg, Germany
2006 –	Head of the Division of Cardiovascular Physiology and Managing Director of the Institute of Physiology and Pathophysiology, University of Heidelberg

Honors

1987-1988	Post-graduate scholarship, Boehringer Ingelheim Fonds
1988-1990	Post-doctoral fellowship, German Research Foundation (DFG)
1991-1993	Lecturer fellowship, German Research Foundation (DFG)
1993	Sandoz Award for Therapy-Related Pharmacological Research, German Society of Experimental and Clinical Pharmacology and Toxicology
1994-1996	Heisenberg fellowship, German Research Foundation (DFG)
2000	Wulf Vater Dihydropyridine Research Award, Wulf Vater-Foundation

Original publications (2001-2007)

1. Lauth M, Cattaruzza M, Hecker M: ACE inhibitor and AT_1 antagonist blockade of deformation-induced gene expression in the rabbit jugular vein through B_2 receptor activation. *Arterioscl Thromb Vasc Biol* 21:61-66, 2001.
2. Lienenlüke B, Stojanovic T, Fiebig T, Fayyazi A, Germann T, Hecker M: Thalidomide impairment of trinitrobenzene sulfonic acid-induced colitis in the rat - Role of endothelial cell-leukocyte interaction. *Br J Pharmacol* 133:1414-1423, 2001.
3. Wagner AH, Schroeter MR, Hecker M: 17β-Estradiol inhibition of NADPH oxidase expression in human endothelial cells. *FASEB J* 15:2121-2130, 2001.
4. Cattaruzza M, Eberhardt I, Hecker M: Mechanosensitive transcription factors involved in endothelin B receptor expression. *J Biol Chem* 276:36999-37003, 2001.
5. Cattaruzza M, Berger MM, Ochs M, Fayyazi A, Füzesi L, Richter J, Hecker M: Deformation-induced endothelin B receptor-mediated smooth muscle cell apoptosis is matrix-dependent. *Cell Death Diff* 9:219-226, 2002.
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7. Stojanovic T, Bedke J, Gröne HJ, Proudfoot AEI, Becker H, Markus P, Hecker M: Met-RANTES inhibition of mucosal perfusion failure in acute intestinal transplant rejection - role of endothelial cell-leukocyte interaction. *J Vasc Res* 39:51-58, 2002.
8. Cattaruzza M, Schäfer K, Hecker M: Cytokine-induced down-regulation of zfm1/splicing factor-1 promotes smooth muscle cell proliferation. *J Biol Chem* 277:6582-6589, 2002.
9. Buchwald AB, Wagner AH, Webel C, Hecker M: Decoy oligodeoxynucleotide against activator protein-1 reduces neointimal proliferation after coronary angioplasty in hypercholesterolemic minipigs. *J Am Coll Cardiol* 39:732-738, 2002.
10. Schramm L, La M, Heidbreder E, Hecker M, Beckmann J, Lopau K, Zimmermann J, Rendl J, Reiners C, Winderl S, Wanner C, Schmidt HHHW: L-Arginine deficiency and supplementation in experimental acute renal failure and in human kidney transplantation. *Kidney Int* 61:1423-1432, 2002.
11. Wagner AH, Schwabe O, Hecker M: Atorvastatin inhibition of cytokine-inducible nitric oxide synthase expression in native endothelial cells in situ. *Br J Pharmacol* 136:143-149, 2002.
12. Kelkenberg U, Wagner AH, Sarhaddar J, Hecker M, von der Leyen HE: C/EBP decoy oligodeoxynucleotide inhibition of macrophage-rich vascular lesion formation in hypercholesterolemic rabbits. *Arterioscler Thromb Vasc Biol* 22:949-954, 2002.
13. Wagner AH, Gebauer M, Güldenzoph B, Hecker M: 3-Hydroxy-3-methylglutaryl coenzyme A reductase-independet inhibiton of CD40 expression by atorvastatin in human endothelial cells. *Arterioscler Thromb Vasc Biol* 22:1784-1789, 2002.
14. Schaeffer G, Levak-Frank S, Spitaler MM, Osibow K, Malli R, Fleischhacker E, Esenabhalu VE, Wagner AH, Frank S, Hecker M, Graier WF: Intercellular signalling within vascular cells under high D-glucose involves free radical-triggered tyrosine kinase activation. *Diabetologia* 46:773-783, 2003.
15. Cattaruzza M, Slodowski W, Stojakovic M, Krzesz R, Hecker M: Interleukin-10 induction of nitric oxide synthase expression attenuates CD40-mediated Interleukin-12 synthesis in human endothelial cells. *J Biol Chem* 278:37874-37880, 2003.
16. Wagner AH, Güldenzoph B, Lienenlüke B, Hecker M: CD154/CD40-mediated expression of CD154 in endothelial cells - consequences for endothelial cell-monocyte interaction. *Arterioscler Thromb Vasc Biol* 24:715-720, 2004.
17. Cattaruzza M, Lattrich C, Hecker M: The focal adhesion protein zyxin is a mechano-sensitive modulator of gene expression in vascular smooth muscle cells. *Hypertension* 43:726-730, 2004.

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28. Korff T, Aufgebauer K, Hecker M: Cyclic stretch determinates the expression of CD40 in endothelial cells by changing their TGF-β1 response. *Circulation* 116:2288-2297, 2007.

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